

LUD 5793.1 US

CLEAN VERSION

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20/PBS, and were then supplemented with biotinylated, anti-interferon gamma specific antibody at 0.5 ug/ml. After incubation for 2 hours at 37°C, plates were washed, and developed with commercially available reagents, for 1 hour, followed by 10 minutes of incubation with dye substrate. Plates were then prepped for counting, positives being indicated by blue spots. The number of blue spots/well was determined as the frequency of NY-ESO-1 specific CTLs/well.

[0083] Experiments were run, in triplicate, and total number of CTLs was calculated. As controls, one of reagents alone, effector cells alone, or antigen presenting cells alone were used. The difference between the number of positives in stimulated versus non-stimulated cells, was calculated as the effective number of peptide specific CTLs above background. Three peptides were found to be reactive, i.e.:

LLSHGAVIEV (amino acids 102-111 of SEQ ID NO: 23, 158-167 of SEQ ID NO: 32)

SLSKILDTV (amino acids 904-912 of SEQ ID NO: 23, 960-968 of SEQ ID NO: 32)

SLDQKLFQL (amino acids 1262-1270 of SEQ ID NO: 23, 1318-1326 of SEQ ID NO: 32).

[0084] The complete list of peptides tested, with reference to their position in SEQ ID NO: 23, follows:

Peptide	Position
FLVDRKVCQL	amino acids 35-43 of SEQ ID NO: 23
ILIDSGADI	amino acids 68-76 of SEQ ID NO: 23
AVYSEILSV	amino acids 90-98 of SEQ ID NO: 23
ILSVVAKLL	amino acids 95-103 of SEQ ID NO: 23
LLSHGAVIEV	amino acids 102-111 of SEQ ID NO: 23
KLLSHGAVI	amino acids 101-109 of SEQ ID NO: 23
FLLIKNANA	amino acids 134-142 of SEQ ID NO: 23
MLLQQNVDV	amino acids 167-175 of SEQ ID NO: 23
GMLLQQNVDV	amino acids 166-175 of SEQ ID NO: 23
LLQQNVDVFA	amino acids 168-177 of SEQ ID NO: 23

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IAWEKKETPV	amino acids 361-370 of SEQ ID NO: 23
SLFESSAKI	amino acids 430-438 of SEQ ID NO: 23
CIPENSIYQKV	amino acids 441-450 of SEQ ID NO: 23
KVMEINREV	amino acids 449-457 of SEQ ID NO: 23
ELMDMQTFKA	amino acids 687-696 of SEQ ID NO: 23
ELMDMQTFKA	amino acids 806-815 of SEQ ID NO: 23
SLSKILDTV	amino acids 904-912 of SEQ ID NO: 23
KILDTVHSC	amino acids 907-915 of SEQ ID NO: 23
ILNEKIREEL	amino acids 987-996 of SEQ ID NO: 23
RIQDIELKSV	amino acids 1018-1027 of SEQ ID NO: 23
YLLHENCML	amino acids 1043-1051 of SEQ ID NO: 23
CMLKKEIAML	amino acids 1049-1058 of SEQ ID NO: 23
AMLKLELATL	amino acids 1056-1065 of SEQ ID NO: 23
KILKEKNAEL	amino acids 1081-1090 of SEQ ID NO: 23
VLIAENTML	amino acids 1114-1122 of SEQ ID NO: 23
CLQRKMNV DV	amino acids 1174-1183 of SEQ ID NO: 23
KMNV DVSST	amino acids 1178-1186 of SEQ ID NO: 23
SLDQKLFQL	amino acids 1262-1270 of SEQ ID NO: 23
KLFQLQSKNM	amino acids 1266-1275 of SEQ ID NO: 23
FQLQSKNMWL	amino acids 1268-1277 of SEQ ID NO: 23
QLQSKNMWL	amino acids 1269-1277 of SEQ ID NO: 23
NMWLQQQLV	amino acids 1274-1282 of SEQ ID NO: 23
WLQQQLVHA	amino acids 1276-1284 of SEQ ID NO: 23
KITIDIHFL	amino acids 1293-1301 of SEQ ID NO: 23

EXAMPLE 22

[0085] Expression of the full length NY-BR-1 molecule was analyzed, by determining the presence of mRNA, in various normal and tumor tissue samples.

[0086] RT-PCR assays were carried out, as described in examples 5 & 9, on a variety of tissue samples.